

**Co-spread of metal and antibiotic resistance within ST3-IncHI2 plasmids
from *E. coli* isolates of food-producing animals**

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Table S1 Primers used for PCR and sequence analysis

PCR	Primer ^a	Primer sequence (5' → 3')	PCR product length(bp)	Target	Reference
A	A ^F	CCACTGCGCGGAATTCCACTCACCAT	231	screening for <i>terD</i>	(1)
	A ^R	ACGCCGTCCCGTCTGATGTTGACAAG			
B	B ^F	CCGACAAACTTCCAGAACAGATGGGGTAGT	428	screening for <i>terF</i>	(1)
	B ^R	GAGGCAGCGGTTGCATTGTACTTGACG			
C	C ^F	ATGCGCCGCCCTGCCTGTTACCTTGTAA	576	screening for <i>terX</i>	(1)
	C ^R	CGCGCTTGTGCTGCCGGAAAGACA			
D	D ^F	CCTGGGGCCGTCAGCGGACCTG	302	screening for <i>terY3</i>	(1)
	D ^R	TCCTTGCTGGTGGCCGTTCATACTTCAT			
E	E ^F	CATGCCCTTGTGATGACGAT	575	screening for <i>merA</i>	This study

	E ^R	GCAATTCA GCCATCACCGTCC			
	F ^F	GGCCGACATAGAGCAGTCCAC			
F	F ^R	CGCATCGCTGGCAAAACCG	313	screening for <i>merC</i>	This study
	G ^F	AGTGAAAGACAGACGAAGCG			
G	G ^R	GGCAGATA GTGTGGAATGCG	244	screening for <i>arsB</i>	(2)
	H ^F	GAAGGACGAACAGCACCTTC			
H	H ^R	CGCATCTGATTACAGCATT	276	screening for <i>arsH</i>	(3)
	I ^F	ATCCGGAAAGGT CAGCACCGTCCATAGAC			
I	I ^R	GACCTCGCGGATGTCAGTGGCTACACCT	507	screening for <i>pcoA</i>	(1)
	J ^F	GGCGCCCAGAATGATAATCGCAACA			
J	J ^R	GGGCGTGGCGCTGGCTACACTT	502	screening for <i>pcoD</i>	(1)

K	K ^F	GTGGGGCAGCTTGCTCAGTCCAGTGA			
	K ^R	CGAAGCTTCTTGCCTGCGTCTGATGTG	385	screening for <i>pcoE</i>	(1)
L	L ^F	TCGGCCTGGGCCACTGAAACCGTGAATA			
	L ^R	GGCGGTGCGCTTCGGCCATAGCCTGATG	364	screening for <i>silE</i>	(1)
M	M ^F	ACACCCCCGGCCTGGGCTCCTT			
	M ^R	TGCGGGCACGGGAACAAACCTC	603	screening for <i>silP</i>	(1)
N	N ^F	AAATGAATGGCTCTGCTCGGTA			
	N ^R	ATTTCCTCTATCATCGCCTCT	585	screening for <i>hipA</i>	This study
O	O ^F	CCGCCTCGCTATATACTTCCG			
	O ^R	CACTAAAGGAATGGCGAAAGCTG	240	screening for <i>relE</i>	This study
P	P ^F	TTGATTCAAGAACACCCGGAT			
	P ^R	TATCTCATTAAAGGCGCTGACC	597	screening for <i>mucB</i>	This study
Q	Q ^F	CTATCCGTACAAGGGAGTGT	~1600	junction between the 5' end of	(4)

	Q ^R	TTTCCACTCGCCTTCACC		ISEcp1 and 3' end of IS903	
R	R ^F	CTATCCGTACAAGGGAGTGT	~ 1300	junction between the 5' end of	(4)
	R ^R	CAGCGGAAGGAGAACCGAG		ISEcp1 and 3' end of orf477	
S	S ^F	ATGAACCCATTCAAAGGCCG	~ 4500	junction between the 5' end of	(5) (6)
	S ^R	GCGGTGCTGTCGATTAA		IS26 and 5' end of oqxB	
T	T ^F	TTACATTCAAAAACTCTGCTTACC		junction between the 3' end of	
T	T ^R	TACCGGAACCCATCTCGAT	~ 3000	IS26	(5) (6)
				and 3' end of oqxB	
U	U ^F	CCTGCCGAATGGGATGAAT	~ 1800	inverse PCR amplification of the IS26-oqxAB- IS26 region	This study
	U ^R	CGCTTCAATGCGACCGTTA			

^aF, forward primer; R, reverse primer.

References

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Table S2 Primers used for PCR and sequence analysis

Primer	Sequence (5' to 3', as synthesized)	Expected amplicon size (bp)	Target	Nucleotide positions	GenBank accession no. ^a
A-1F	CGGCTTACCCAACAAATAGCAA	6564	junction between <i>tnsA</i> and conserved hypothetical protein	161249-161270	KT347600
A-1R	ATCATCAAGTCCGATAGAGGCAT			154707-154729	
B-2F	GATTATCATTACTGGGAGCCTA	6943	junction between <i>tnsB</i> and <i>cuss</i> (<i>silS</i>)	156325-156346	KT347600
B-2R	CACCTCCGAGAATCTTGATGC			149404-149424	
C-3F	GAAACGGTCTGACTTCCCAT	5499	junction between <i>silE</i> and <i>silB</i>	150729-150748	KT347600
C-3R	CTGAATCTGAGCCACCACCT			145249-145268	
D-4F	CTATTCCCGACAGGTTAACGAA	8409	junction between <i>silC</i> and cell wall endopeptidase	146527-146548	KT347600
D-4R	CCCGCTGATAACAAACTCGAAC			138098-138119	
E-5F	GGATAACGCAATAGCACCTCC	3668	junction between cell wall endopeptidase and <i>copB</i> (<i>pcoB</i>)	137984-138004	KT347600
E-5R	CAGTTGATCCAGCAGAACCCAG			134337-134357	
F-6F	CGTATCACTGCCATTGCTCT	4925(2644)	junction between <i>copA</i> (<i>pcoA</i>) and <i>copE</i> (<i>pcoE</i>)	134686-134709	KT347600
F-6R	CATTCAGCCATGTCCTGGTG			132066-132086	
G-7F	CAGTCTCCGTTGTTACCA	5656	junction between <i>pcoE</i> and <i>dcm</i>	131932-131951	KT347600
G-7R	ACAGCTTCGACCATTATGACC			126294-12315	

H-8F	ATTACGTGCCGAAGTATCCAG	5588	junction between <i>tnpA</i> and <i>copE</i>	1265-1285	KU248943
H-8R	TTATGCATTCAGCCATGTCC			7606-7626	
I-9F	CTGGCCCCTTAGTAACGAG	3055	junction between putative peptidase and <i>insL</i>	23760-23779	KU248944
I-9R	ACATTATCTGACGTGGCTCT			26795-26814	

^aSequence used for primer design.

Table S3. Exploration genetic context of the *pco* and *sil* genes by PCR mapping.

Plasmids	GenBank accession no.	PCR								
		A-1	B-2	C-3	D-4	E-5	F-6	J-7	H-8	I-9
pEC5027	KT347600	+	+	+	+	+	+	+	-	-
pS151T	This study	+	+	+	+	+	+	+	-	-
pFS7Z5GT	This study	+	+	+	+	+	+	+	-	-
P3YG7T	This study	+	+	+	+	+	+	+	-	-
pZ13T	This study	+	+	+	+	-	+	+	+	+
pA84T	This study	+	+	+	+	-	+	+	+	+

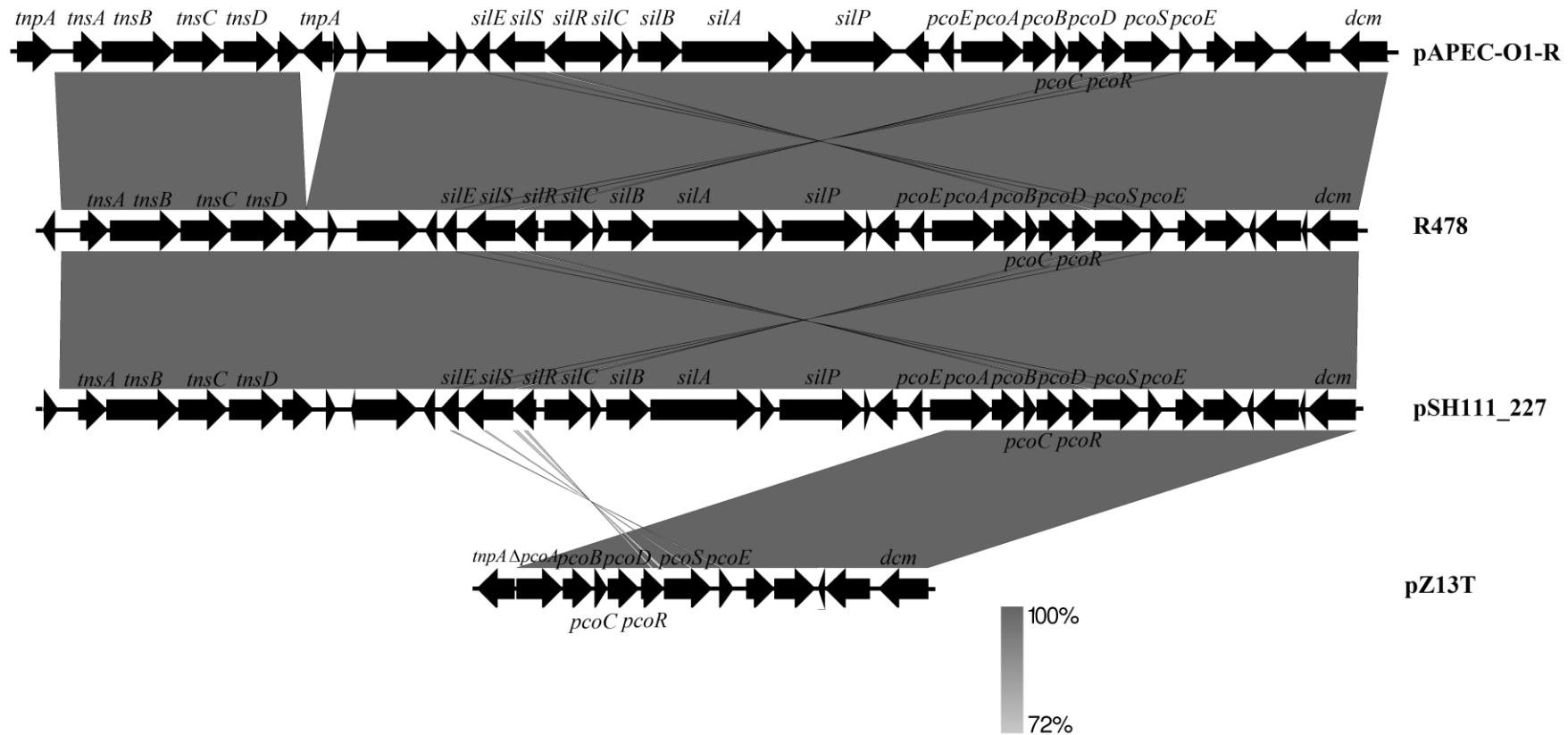


Fig S1 Characteristic of the genetic contexts of the *pco* operons. Plasmids pAPEC-O1-R (BX663045), R478 (DQ517526), and pSH111_227(JN983042) were from GenBank; and pZ13T (KU248943) were from this study.

Fig S2A

		Percent Identity							
		1	2	3	4	5	6	7	
Divergence	1	97.8	95.1	100.0	99.4	100.0	99.4	1	pAPEC-O1-R (BX663045)
	2	2.2	96.3	97.8	98.3	97.8	98.3	2	pEC5027 (KT347600)
	3	5.0	3.8	95.1	95.1	95.1	95.0	3	pMG101 (AF067954)
	4	0.0	2.2	5.1	99.4	100.0	99.4	4	pSH111-227 (JN983042)
	5	0.6	1.7	5.1	0.6	99.4	100.0	5	pZ13T (KU248944)
	6	0.0	2.2	5.1	0.0	0.6	99.4	6	R478 (DQ517526)
	7	0.6	1.7	5.1	0.6	0.0	0.6	7	p3YG7T (KU248945)
		1	2	3	4	5	6	7	

Fig S2B

		Percent Identity							
		1	2	3	4	5	6	7	
Divergence	1	85.2	86.3	82.6	86.4	86.3	86.3	1	pEC5027 (KT347600)
	2	16.5	99.4	99.4	99.4	99.4	99.4	2	pRJ1004 (X83541)
	3	15.2	0.6	99.9	99.9	99.9	99.9	3	pSH111_227 (JN983042)
	4	19.9	0.6	0.1	100.0	100.0	100.0	4	pZ13T (KU248943)
	5	15.1	0.6	0.1	0.0	99.9	100.0	5	R478 (DQ517526)
	6	15.2	0.6	0.1	0.0	0.1	99.9	6	p3YG7T (KU248945)
	7	15.1	0.6	0.1	0.0	0.0	0.1	7	pAPEC-O1-R (BX663045)
		1	2	3	4	5	6	7	

Fig S2 DNA sequence alignment of the *sil* or *pco* operons by using the DNASTAR software MagAlign Program. A) Alignment of the nucleotide sequence of the *sil* operon (based on *sil*ESRCBAP sequence); B) Alignment of the nucleotide sequence of the *pco* operon (based on *pco*EABCDRSE sequence). The sequence were from plasmids pAPEC-O1-R (BX663045), R478 (DQ517526), pSH111_227(JN983042), pEC5207 (KT347600), pMG101 (AF067954), PRJ1004 (X83541) p3YG7T (this study) and pZ13T (this study).